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PERSPECTIVE

Intracellular wetting mediates contacts between liquid compartments and membrane-bound organelles

Halim Kusumaatmaja^{1*} , Alexander I. May^{2,3*} , and Roland L. Knorr^{4,5*} 

Protein-rich droplets, such as stress granules, P-bodies, and the nucleolus, perform diverse and specialized cellular functions. Recent evidence has shown the droplets, which are also known as biomolecular condensates or membrane-less compartments, form by phase separation. Many droplets also contact membrane-bound organelles, thereby functioning in development, intracellular degradation, and organization. These underappreciated interactions have major implications for our fundamental understanding of cells. Starting with a brief introduction to wetting phenomena, we summarize recent progress in the emerging field of droplet-membrane contact. We describe the physical mechanism of droplet-membrane interactions, discuss how these interactions remodel droplets and membranes, and introduce "membrane scaffolding" by liquids as a novel reshaping mechanism, thereby demonstrating that droplet-membrane interactions are elastic wetting phenomena. "Membrane-less" and "membrane-bound" condensates likely represent distinct wetting states that together link phase separation with mechanosensitivity and explain key structures observed during embryogenesis, during autophagy, and at synapses. We therefore contend that droplet wetting on membranes provides a robust and intricate means of intracellular organization.

Cells as three-compartment systems

The historical paradigm that cellular organization comprises two key structural components—the cytosolic material and membrane-bound compartments—has recently been challenged by the emerging importance of non-membrane-bound compartments. These proteinaceous compartments have been variously referred to as biomolecular condensates, membrane-less organelles, and biomolecular droplets (herein "droplets"). Such droplets play a diverse range of roles in the cell. Examples include stress granules (made up of RNAs and RNA-binding proteins that phase-separate to sequester translationally stalled ribonucleoproteins during periods of stress; [van Leeuwen and Rabouille, 2019](#)), the nucleolus (an intra-nuclear structure comprising proteins and nucleic acids that is involved in ribosome biogenesis and stress response; [Brangwynne et al., 2011](#); [Feric et al., 2016](#)), and P-bodies (proteinaceous droplets important for RNA processing in the cytosol; [Marnik and Updike, 2019](#); [van Leeuwen and Rabouille, 2019](#)). Droplets form in the cytosol and nucleolus through liquid-liquid phase separation, a demixing process that generates liquid-like compartments with

physicochemical properties distinct from the surrounding solution ([Alberti, 2017](#); [Banani et al., 2017](#); [Brangwynne et al., 2009](#); [Shin and Brangwynne, 2017](#)). The functional diversity, dynamics, and lack of enclosing membranes are unique features of droplets that have ground-breaking implications for our understanding of cellular organization.

The complexity of droplets is particularly relevant when studying droplet dynamics. A key feature of droplets is that they can be both viscous (a hallmark of liquids) and elastic (as observed in solids), a phenomenon known as viscoelasticity ([Bergeron-Sandoval and Michnick, 2018](#)). Whether droplets behave more like a solid or a liquid depends on a range of parameters including the mechanical stress applied, droplet age and size, and time. Importantly, it has been suggested that changes in the material properties of droplets can be attributed to perturbations in autophagy ([Yamasaki et al., 2020](#)), are linked to aging ([Alberti and Hyman, 2016](#)), and are also associated with various diseases, including neurodegenerative pathologies such as Alzheimer's disease and amyotrophic lateral sclerosis ([Alberti and Hyman, 2016](#); [Patel et al., 2015](#)).

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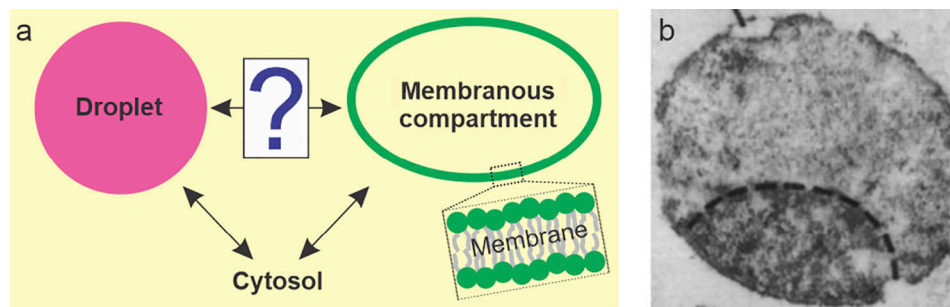


Figure 1. Three classes of intracellular interfaces. (a) The cytoplasm of cells contains membrane-bound and droplet compartments as well as the cytosol. Accordingly, three major classes of intracellular interface exist. This review focuses on contacts between droplets and membranes. We use yellow to indicate the cytosol, magenta for droplets, and green for membranes (and other substrates) throughout this review. (b) Very early evidence for contacts between droplets and membranes in yeast cells. A section through a nucleus is shown as imaged by electron microscopy ($\times 12,000$). The electron-dense material is the nucleolus, which we now understand to be the major droplet subcompartment of the nucleus. The nucleolus forms a lens-like shape as a result of its wetting on the membrane of the nuclear envelope. b is reprinted with permission from Elsevier from Molenaar et al. (1970).

Potential cytosol-droplet and droplet-membrane interactions are therefore of great importance in understanding sub-cellular dynamics (Fig. 1 a). While the effect of cytosol-droplet interactions on cellular organization and physiology has been established (reviewed in Alberti et al., 2019; Banani et al., 2017; Shin and Brangwynne, 2017), the mechanism underlying droplet-membrane interactions remains poorly understood. The functional importance of droplet-membrane interactions has only very recently been shown, for example in T cell receptor signal transduction (Su et al., 2016), pre- and postsynaptic densities (Milovanovic et al., 2018; Zeng et al., 2018), the development of tight junctions that control paracellular transport (Beutel et al., 2019), droplet transport by hitchhiking on moving lysosomes (Liao et al., 2019), the assembly of membranes implicated in autophagy (Fujioka et al., 2020; Agudo-Canalejo et al., 2021), endoplasmic reticulum-mediated droplet splitting (Lee et al., 2020), and formation of protein storage vacuoles (Kusumaatmaja et al., 2021). These examples demonstrate that referring to droplets as “membrane-less organelles” is misleading as, in fact, droplets frequently interact with several distinct classes of membrane: the plasma membrane and membrane-bound organelles as well as small vesicular compartments and membrane sheets.

In this perspective, we propose that droplet-membrane interactions can be generalized as a wetting phenomenon. Below, we discuss the current understanding of intracellular wetting, focusing on the impact of wetting on membranes, and provide several fundamental examples of droplet-membrane interplay. Finally, we describe regulatory mechanisms that control the formation of contact sites between droplets and membranes. We contend that accumulating evidence indicates that wetting provides a robust and carefully balanced means of intracellular organization.

Three states of droplet-surface interactions: Complete wetting, partial wetting, and dewetting

The discovery that P-bodies are droplets with features akin to liquid-like materials (Brangwynne et al., 2009) was followed closely by the identification of numerous additional cytosolic compartments that are now considered droplets (Alberti et al., 2019; Banani et al., 2017; Shin and Brangwynne, 2017). Droplets

have also been identified within membrane-bound organelles; the nucleolus is one prominent example of an intraorganellar compartment that is characterized by liquid-like mechanical properties (Brangwynne et al., 2011). Among such droplet properties is droplet surface tension, which results from cohesive forces within the droplet and drives droplets to minimize their surface area. Surface tension allows small, nonadhering droplets to assume a spherical morphology, and is also behind the shape relaxation observed in coalescing droplets (Alberti et al., 2019; de Gennes, 1985). The liquid-like properties of droplets allow for droplet deformation into spherical caps when a droplet contacts a substrate, a phenomenon known as wetting. An overview of the literature reveals that wetting structures were first observed in cells half a century ago: electron microscopy of isolated yeast nuclei clearly shows the nucleolus adhering to the membrane of the nuclear envelope (Molenaar et al., 1970). Notably, the images presented in this study show a nucleolus deforming into a lens-like shape in response to membrane adhesion, indicative of nucleolar wetting (Fig. 1 b).

Theoretically, wetting is best understood using a simple scenario in which a droplet is in contact with a smooth, flat, and rigid substrate (Fig. 2). The shape of the droplet is determined by the balance between three interfacial energies, which are associated with the droplet-substrate (γ_{ds}), droplet-cytosol (γ_{dc} , also known as droplet surface tension), and cytosol-substrate (γ_{cs}) interfaces. The total energy is given by

$$E = \gamma_{ds}A_{ds} + \gamma_{dc}A_{dc} + \gamma_{cs}A_{cs}, \quad (1)$$

where each term is proportional to the interfacial area (denoted by A), and the constant of proportionality is called the surface or interfacial tension (denoted by γ). An interface is less favorable the higher its interfacial tension value; accordingly, the droplet relaxes its deformation to minimize the total energy.

Depending on the relative magnitude of interfacial tensions, one of three wetting states will arise in this scenario. First, if the droplet-substrate interaction is particularly energetically favorable in comparison with the cytosol-substrate interaction ($\gamma_{cs} > \gamma_{ds} + \gamma_{dc}$), the droplet will spread to form a film on the substrate (Fig. 2 a). This is termed a complete wetting state. On the other hand, if the droplet-substrate interaction is very

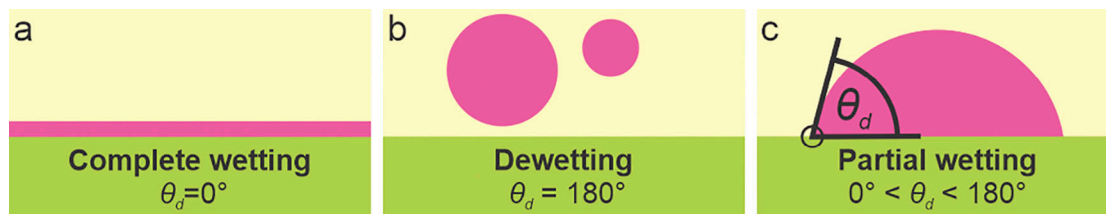


Figure 2. Wetting of droplets on a rigid substrate. (a) The complete wetting state occurs with the formation of a film on the surface of the substrate when the droplet–substrate interaction is favorable. (b) In contrast, when droplet–substrate interactions are not favorable, a dewetting state is realized in which the droplet remains completely unassociated with membrane. (c) A droplet contact angle, or Young’s contact angle θ_d , characterizes partial wetting states. Partial wetting occurs when droplet–substrate and droplet–cytosol interactions are comparable. A three-phase contact line between the deformed droplet, the substrate, and the surrounding solution forms (indicated by the circle).

unfavorable ($\gamma_{ds} > \gamma_{cs} + \gamma_{dc}$), the droplet does not interact with the substrate at all, a situation known as dewetting (Fig. 2 b). When droplet–substrate and cytosol–substrate interactions are comparable, a partial wetting state is achieved (Fig. 2 c). Such interactions are characterized by a droplet–substrate contact angle, θ_d , that is often termed the Young’s contact angle (Fig. 2). The Young’s contact angle is typically used as measure of wettability and represents the balance of the three interfacial tensions at the three-phase contact line:

$$\cos\theta_d = \frac{\gamma_{cs} - \gamma_{ds}}{\gamma_{dc}}. \quad (2)$$

This contact angle can strongly affect the formation and behavior of droplets. For example, the density fluctuation of liquids increases in the vicinity of a solid substrate, which allows droplets to nucleate more easily on wetting substrates compared with nonwetting substrates (Kalikmanov, 2013; Kurotani and Tanaka, 2020). Wettability is therefore a key determinant of droplet formation and behavior on a substrate.

Contact sites between droplets and membranes represent partial wetting states

Of course, wetting is not limited to rigid substrates. Biological settings in particular are characterized by a large variety of soft elastic substrates (Kasza et al., 2007; Pegoraro et al., 2017) that may undergo deformation as a direct result of a partially wetting droplet. This interplay between substrate elasticity and droplet surface tension is called elastocapillarity (Style et al., 2017; Bico et al., 2018). The characteristic length scale over which elastocapillary phenomena are likely to be observed is determined by comparing the energy scales for elastic substrate deformation and droplet surface tension. In this manuscript, we focus on the case where the elastic substrate corresponds to lipid membranes (Fig. 3). Membranes are very easy to deform: their elasticity is similar to that of a very fine rubber sheet of comparable thinness (~ 4 nm; Gracià et al., 2010). Membrane deformation usually results in bending of the membrane, with the energy scale of this deformation corresponding to κ , a measure of membrane bending rigidity. For the surface tension energy, it scales as $\gamma_{dc}l^2$, where l is the typical droplet size. Equating both energy scales leads to the elastocapillary length $l \sim (\kappa/\gamma_{dc})^{1/2} \sim 0.1 - 10 \mu\text{m}$ (Kusumaatmaja and Lipowsky, 2011; Kusumaatmaja et al., 2009). Here, we have used typical values for the bending rigidity,

$\kappa = 10^{-20} - 10^{-18} \text{ J}$ (Gracià et al., 2010) and droplet surface tension, $\gamma_{dc} = 1 - 100 \mu\text{N/m}$ (Aumiller and Keating, 2017; Jawerth et al., 2018). Intriguingly, the elastocapillary length is within the size range of the majority of membrane structures and droplets in cells, suggesting that cells are likely to have evolved mechanisms to harness elastocapillary forces in diverse biological pathways.

For droplets that wet membranes partially, we previously showed that the droplet and membrane deformation concentrates at the three-phase contact line, and that contact angles can be measured using a simple spherical cap approximation (Kusumaatmaja et al., 2009). Either of two conditions is required to achieve such relatively simple geometries. The first condition is a tense membrane, which is akin to the rigid substrate case (Fig. 2). The cytosol and substrate form the contact angles $\theta_c \cong 180^\circ - \theta_d$ and $\theta_s \cong 180^\circ$, respectively (see Fig. 3, a and d). The second condition is a membrane with low tension and a droplet size that is considerably larger than the elastocapillary length (Fig. 3 b). Such simple spherical cap geometry is reminiscent of the shapes observed when two or more droplets interact (Feric et al., 2016). However, there are key differences. For droplet–droplet interactions, all surface tensions are material parameters that depend only on the compositions of the droplets. In contrast, the material parameters of droplet–membrane interactions are the Young’s contact angle (θ_d) and droplet–cytosol surface tension (γ_{dc}), while the membrane tensions (γ_{ds} and γ_{cs}) can be mechanically varied. These factors can bring the elastocapillary length into a similar range as the droplet size; this is important when low tension membranes wet droplets of a comparable size to the elastocapillary length, which results in mutual droplet–membrane remodeling and complex geometries (Fig. 3 c). These factors explain why droplet and membrane shapes are sensitive to the fundamental characteristics of the system, such as droplet size and membrane tension. A key biological implication of these underlying factors is that droplet–membrane contacts are sensitive to mechanical perturbations that alter membrane tension, for example, as induced by osmotic stress.

In vitro studies have confirmed that polymeric droplets can partially wet and remodel membranes. These experiments employ artificial model systems, whereby two immiscible polyethylene glycol–dextran solutions coexist inside micrometer-sized lipid vesicles known as giant unilamellar vesicles (Long et al., 2005). Under these conditions, a partially wetting droplet forms a lens-like shape that is comparable to wetting nucleoli (Fig. 1 b and

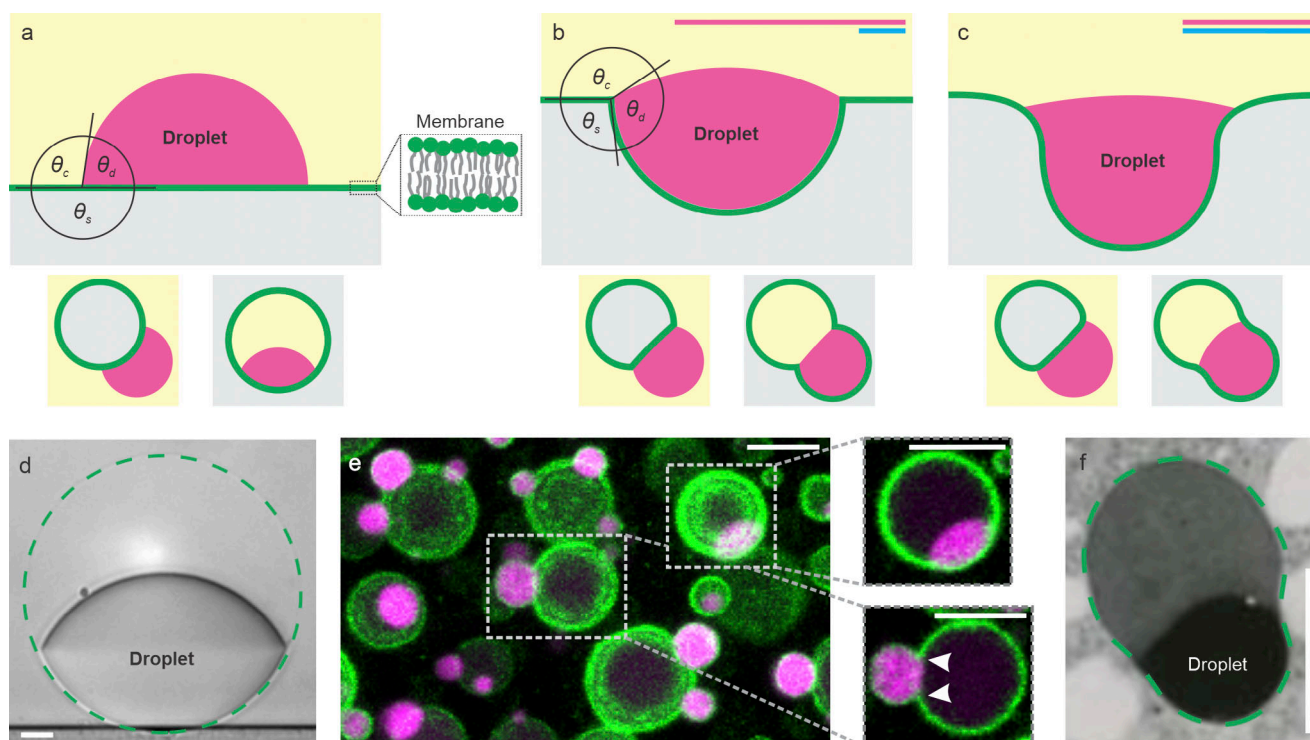


Figure 3. Partially wetting droplets act as a liquid scaffold that deforms membranes. (a) Tense membranes do not deform in response to a wetting droplet. The upper panel depicts a wetting interaction between a droplet and a flat membrane with $\theta_s \cong 180^\circ$. The lower panels illustrate geometries of vesicles made up of tense membranes wetted by droplets externally (left) or internally (right). (b and c) Droplets act as liquid scaffolds that deform wetted membranes with low tension. The ratio between droplet size and elastocapillary length determines the complexity of the droplet–membrane deformation. Droplets shown in b are considerably larger than the elastocapillary length and form a simple spherical cap geometry. Droplets shown in c are comparable in size to the elastocapillary length and create a more complex droplet–membrane morphology. The colored scale bars in b and c are provided as a guide for the ratio between droplet size (magenta) and elastocapillary length (blue). (d) In vitro morphology of a wetting polymer droplet enclosed within a micrometer-sized vesicle (green broken line; the horizontal line is the base of observation chamber; Liu et al., 2016). This geometry is analogous to that shown in (a) and Fig. 1 b. Scale bar: 10 μm . (e) Confocal images of a live *Arabidopsis thaliana* embryo showing interior wetting morphologies within vacuoles, as performed in Feeney et al. (2018). Single sections that correspond to a and b are magnified. Wetting droplets remodel vacuolar membranes that form membrane buds (arrowheads: membrane neck). TPK1-GFP fluorescence was used to visualize membranes. Scale bar: 5 μm . (f) High-resolution image of a plant vacuole enclosing a wetting droplet (Zheng and Staehelin, 2011), corresponding to the geometry shown in c. A broken green line denotes the vacuolar membrane. Scale bar: 2 μm . Data are a maximum-intensity projection of confocal sections (e, left), magnified single confocal sections (e, right), and an electron microscope image (f). d is reprinted with permission by the American Chemical Society (Liu et al., 2016; <https://pubs.acs.org/doi/10.1021/acsnano.5b05377>). f is reprinted by permission of Oxford University Press (Zheng and Staehelin, 2011).

Fig. 3 d). Subsequent studies have reported similar droplet–membrane wetting for a broad range of droplets and membranes in vitro. These studies used molecules that phase-separate by a mechanism distinct from polyethylene glycol–dextran (non-associative versus associative phase separation; Poudyal et al., 2018) and nonlipidic membranes, as well as membrane-bound organelles (chloroplasts; Last et al., 2020; Pavan Kumar et al., 2018; Martin et al., 2018). Taken together, these results suggest that wetting is a general feature of droplet–membrane interactions. In addition, these studies demonstrate the value of in vitro models in studying wetting phenomena: such models allow fine-tuning of the physiochemical properties of compartments, which is critical as we improve our understanding of droplet–membrane wetting parameters.

Liquid scaffolding mediates remodeling of embryonic vacuoles in plants

A hallmark of seed maturation in plant embryos is the remodeling and division of preexisting vacuoles, which are generally

the largest single membrane-bound organelle in plant cells, into multiple protein storage vacuoles as these organelles accumulate storage proteins (Feeney et al., 2018). While the mechanism that drives this transition is unclear, the diverse shapes of vacuoles during embryogenesis very closely resemble the wetting morphologies discussed above (compare Fig. 3, e and f, with Fig. 1 b and Fig. 3, a–d). In fact, at specific developmental stages, vacuolar storage protein subcompartments form and remodel vacuolar membranes at their three-phase contact line. This observation suggests that vacuolar subcompartments are phase-separated droplets that wet the vacuolar membrane and act as a liquid scaffold that mediates membrane deformation, eventually resulting in the formation and scission of storage protein droplet-enclosing membrane buds. Further, it implies that elastocapillarity (the interplay of inherent material attributes of both the subcompartment and the vacuole membrane, respectively droplet surface tension and membrane elasticity) governs protein storage vacuole formation. To distinguish this

droplet-mediated mechanism of cellular membrane reshaping from conventional membrane remodeling complexes that form membrane coats such as clathrin (Avinoam et al., 2015), we refer to this novel form of droplet-mediated membrane remodeling as “liquid scaffolding.” Liquid scaffolding during vacuole remodeling has been confirmed recently (Kusumaatmaja et al., 2021).

Droplet transport by partial wetting on mobile membrane-bound organelles

Evidence also suggests that wetting plays a role in the intracellular relocation of liquids. In cells, droplets are repositioned by several mechanisms, including controlled phase dissolution/phase separation, cytoplasmic flow, and active transport by motor proteins (Brangwynne et al., 2009; Liao et al., 2019; Mittasch et al., 2018). Motor proteins are especially important for long distance movements, such as transport from the cell body to the terminal of a neuron. The question of how liquid droplets interact with motor proteins has been addressed in recent research, which revealed that a membrane-bound organelle is critical in mediating droplet-motor protein interactions: RNA-containing droplets in fact hitchhike on lysosomes that are themselves undergoing transport (Liao et al., 2019). Intriguingly, images published in this study show that lysosome-bound RNA droplets deform into spheroidal caps consistent with droplets wetting the exterior of an organelle (Fig. 3 a), again suggesting a critical role for partial wetting. Droplet transport therefore demonstrates yet another potential function of wetting, underscoring the fundamental integration of intracellular wetting into cell physiology.

Wetting droplets can form complex morphologies

Membrane-bound droplets have been found to underlie the assembly of several adhesion complexes (Beutel et al., 2019; Su et al., 2016). A detailed examination of tight junctions revealed that droplets interacting with the plasma membrane are critical for tight junction formation. Such droplets bind the plasma membrane from the cytosolic side, forming a perimeter around the entire circumference of the cell that separates apical and basolateral membranes. Such droplet-plasma membrane contacts occur via multivalent interactions mediated by a conserved, phosphorylation-sensitive domain within the phase separating tight junction zonula occludens (ZO) proteins. Consequently, key tight junction proteins, including polymerizable adhesion receptors of the claudin protein family, cytoskeletal adapters, and transcription factors that are important for tight junction establishment, were reportedly enriched in ZO droplets (Beutel et al., 2019). These results imply that tight junctions might act as intercellular bridges between plasma membrane-wetting ZO droplets that form within cells and are “reinforced” by claudin fibers.

Formation of domain-like droplet thin films on membranes

Thus far, we have considered droplet-membrane contacts formed by micrometer-sized droplets where contact angles are clearly observed. An important alternative scenario involves droplet formation that includes cytosolic domains of proteins that are bound to membranes by, for example, transmembrane domains and lipid anchors (reviewed in Case et al., 2019). Under

these conditions, droplets are constrained at the membrane surface. Such droplets therefore cover membranes in the form of thin films (Fig. 4 a). Indeed, recent studies have shown that such droplet films are able to extend laterally over several micrometers (Banjade and Rosen, 2014) and are characterized by smooth, circular boundaries (Yuan et al., 2021). Optically, such thin films made of droplets resemble membranes comprising two coexisting membrane raft-like lipid phases (Vequi-Suplicy et al., 2010; Veatch and Keller, 2003). However, in contrast to coexisting lipid phases, the membranes underneath proteinaceous thin films remain homogeneous. In agreement with recent work on autophagy discussed below (Agudo-Canalejo et al., 2021), Yuan et al. (2021) recently showed that such thin films of droplets can also modulate the spontaneous curvature of membranes (a measure of membrane asymmetry) by observing membrane tube formation from regions wetted by droplet films. Such spontaneous curvature change is a well-established means of generating curved membrane structures in cells (McMahon and Boucrot, 2015), but the mechanism is distinct from that of elastocapillary phenomena. The interplay between both mechanisms can cooperatively orchestrate complex membrane reorganization, as discussed below for the case of droplet-mediated autophagy (Fig. 4 d).

Droplet enclosure by wetting membrane sheets: The mechanism of droplet autophagy

Autophagy is a highly conserved intracellular bulk degradation pathway that captures cellular material for recycling, thereby ensuring survival through the reuse of the cell’s own metabolites. Autophagy is a membranous phenomenon: the double-membrane autophagosome is formed de novo by assembly and expansion of thin, flattened membrane sheets (known as isolation membranes or phagophores), which isolate portions of cytosol (Knorr et al., 2017; Melia et al., 2020; Zhao and Zhang, 2019). Eventually, autophagosomes fuse with lysosomes that contain degrading enzymes to break down the enclosed cargo. Droplet degradation in an autophagy-dependent manner, or “droplet autophagy,” has previously been reported (Buchan et al., 2013; Sun et al., 2018; Zaffagnini et al., 2018; Zhang et al., 2018; Yamasaki et al., 2020), hinting at direct droplet-membrane interactions by wetting. Three sequential steps of droplet autophagy have been described: no enclosure, partial enclosure, and complete enclosure of droplets by autophagosomal membranes (Sun et al., 2018; Danieli and Martens, 2018; Sánchez-Martín and Komatsu, 2018; Wang and Zhang, 2019; Noda et al., 2020). These three steps correspond to dewetting, partial wetting, and complete wetting, the three wetting states discussed above (Fig. 2). Three contact angles characterize the intermediate state (Fig. 4 b), further suggesting that wetting is involved in droplet autophagy.

These initial suggestions of a wetting-like mechanism of droplet sequestration are explained by a minimal physical model of droplet autophagy recently developed by our group (Schultz et al., 2021; Agudo-Canalejo et al., 2021). This model predicts that the three interfacial energies control droplet autophagy (Eq. 1) and that droplets act as liquid scaffolds during autophagosome formation. In addition, our model further suggests that autophagosomal membranes can enclose droplets in a piecemeal

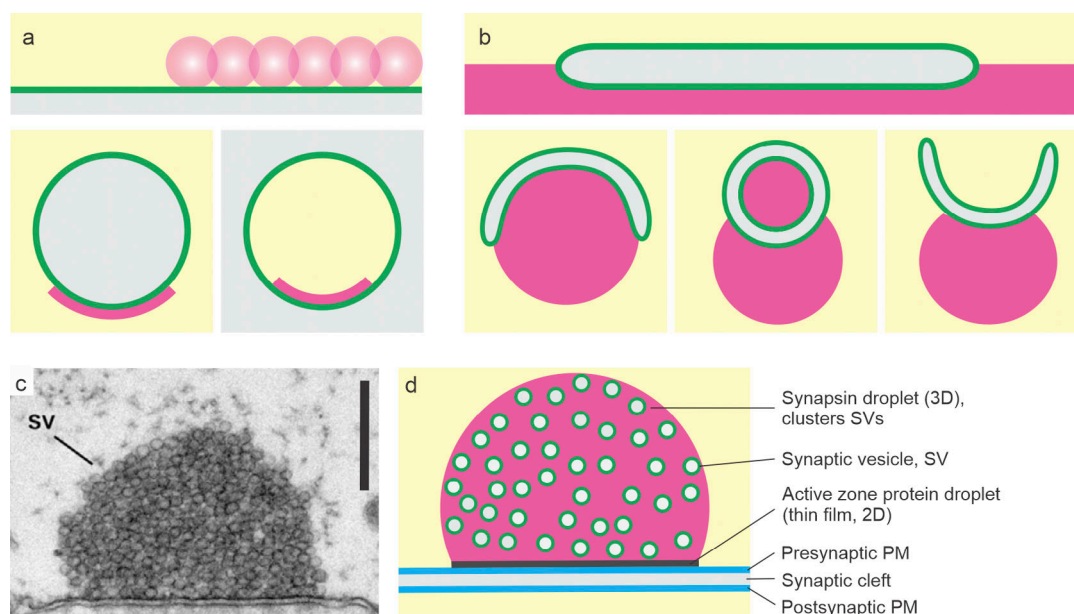


Figure 4. Novel membrane wetting morphologies in cells. (a) Phase separation of membrane-bound proteins results in droplets that spread on the membrane to form a liquid-like thin film. (b) During autophagosome formation in cells, partially wetting membrane sheets can assemble and grow at droplet surfaces. The competition between droplet surface tension and the increasing shape instability of growing membrane sheets that promote sheet bending determines whether a droplet is completely engulfed or isolated in a piecemeal fashion (left and center, lower panels). Further, the membrane spontaneous curvature difference across sheets controls the sheet bending direction and can result in sequestration of the cytosol (right, lower schematic). (c) Droplets mediate vesicle clustering. SV clusters form spherical caps and partially wet the plasma membrane (PM), as visualized by electron microscopy (Pechstein et al., 2020). (d) Schematic of synaptic organization. Green indicates membranes of small SVs clustered in synapsin droplets by complete wetting, whereas light blue denotes both PMs. A thin film formed by a droplet of several active zone proteins wets the presynaptic PM (black line) and defines the localization of the synapsin droplet. Scale bar: 0.5 μm . c is reproduced under a Creative Commons license.

fashion, whereby piecemeal enclosure is induced through either membrane growth or a reduction in droplet surface tension, as confirmed experimentally. Piecemeal autophagy results in droplet splitting, whereby the autophagosome sequesters only one of the two droplets generated (Fig. 4 b). This splitting mechanism is distinct from the droplet splitting mediated by the endoplasmic reticulum that was reported recently (Lee et al., 2020).

Counterintuitively, the bending direction of autophagosomal membrane sheets can also invert to sequester portions of the cytosol while leaving the wetting droplet intact (Fig. 4 b; Schultz et al., 2021; Agudo-Canalejo et al., 2021), a phenomenon confirmed recently (Kageyama et al., 2021). The spontaneous curvature of the membrane sheets controls the process (Agudo-Canalejo et al., 2021). Such droplet-mediated cytosolic autophagy is also consistent with another recent study by our group, which found that in yeast, the site of autophagosome formation (the preautophagosomal structure [PAS]) is in fact a phosphorylation-sensitive droplet comprising autophagy-related proteins (Fujioka et al., 2020). The PAS droplet is not sequestered during the expansion and closure of autophagosomes, suggesting that such an inverted bending mechanism is favored in the case of the PAS. Taken together, these data provide very strong evidence that droplet-mediated autophagy is a complex wetting phenomenon that is controlled by droplet and membrane properties.

Droplet-mediated vesicle clustering

At nerve terminals, vesicles that are filled with neurotransmitter molecules release their contents at active zones into a

narrow intercellular space, the synaptic cleft, to transmit signals from presynaptic to postsynaptic cells. These synaptic vesicles (SVs) are clustered within cap-shaped structures on the plasma membrane, with clusters acting as a reserve pool from which SVs are mobilized during sustained synaptic activity (Fig. 4 c; Pieribone et al., 1995; Milovanovic and De Camilli, 2017). Although this observation suggests that SV clusters partially wet the plasma membrane, how SVs cluster and interact with the plasma membrane is not known.

Recent work has shown that the synaptic protein synapsin phase-separates to form droplets that can internalize numerous small vesicles completely (Milovanovic et al., 2018; Pechstein et al., 2020; Park et al., 2021). This suggests that synapsin droplets cluster SVs through complete membrane wetting (Fig. 2 a and Fig. 4 d). In addition, recent experiments have demonstrated that active zone proteins also phase-separate to form thin droplet films that wet the presynaptic plasma membrane but do not mix with synapsin droplets (Wu et al., 2021, 2019). Importantly, these plasma membrane-wetting active zone droplets appear to define the localization of synapsin-mediated SV clusters (Wu et al., 2021). Together, these results indicate that the nerve terminal is organized by several droplets (Fig. 4 d). This organization is achieved through coexistence of immiscible droplets characterized by distinct droplet-membrane wetting states on SVs as well as the plasma membrane, in addition to droplet-droplet interactions.

Wetting: A regulated means of controlling droplet and membrane behavior

We propose that the phenomenon of wetting is in fact a key means of controlling droplet and membrane behaviors. We have discussed evidence that wetting is a key physical mechanism governing diverse physiological processes within cells, including storage vacuole formation, vesicle clustering, and autophagy. In principle, three wetting states can be distinguished (Fig. 2), each of which has been reported in cells for droplets that interact with membranes. There is also evidence indicating that the transition between the wetting states is biologically important, as observed in the selective sequestration of droplets by membranes during autophagosome formation.

In the literature, droplets are commonly reported in the dewetted state. Such droplets are often described as “membrane-less organelles” as they can organize cellular material in the absence of membranes through phase separation. Meanwhile, droplet behavior is usually explained as determined by constituent proteins. Here, we challenge these conceptions by highlighting that droplets can interact with membranes through both partial and complete wetting states. During partial wetting, droplets, membranes, and the surrounding solution exhibit mutual interactions of comparable strength, which results in dynamic yet persistent contact between the droplet and the membrane, as well as their mutual remodeling. In contrast, the complete wetting state is characterized by a droplet-membrane interaction that exceeds the affinity of a droplet for its surrounding solution. In this case, the droplet fully coats the membrane, internalizes a vesicle-like structure, or is isolated within a membrane. Complete wetting has a range of potential functional implications: full coverage of membranes by droplets reduces the accessibility of the membrane to the surrounding solution or vice versa, and can also cluster vesicles within droplets, allowing for the storage of these vesicles for subsequent use. While studies of biological phase separation have previously focused overwhelmingly on the dewetted membrane-less state, we contend that partial and complete wetting and the transitions between all three states are equally important in the cellular context.

Membrane wetting depends on a range of parameters, including the three interfacial tensions (Eq. 1 and Fig. 3) that are determined by nonspecific (e.g., electrostatic) and specific (e.g., protein-protein) interactions. In general, altering the composition of any component of the system (i.e., droplet, membrane, and cytosol), likely causes a relative change in interfacial tensions. This alters the force balance at the three-phase contact line, in theory causing contact angle changes that can culminate in switching between wetting states. Thus, in addition to spatiotemporal changes in droplet composition (Riback et al., 2020), the physiological state and physicochemical properties of droplets, membranes, and the cytosol that interact are key considerations for our understanding of droplet dynamics and their role in intracellular wetting.

There are a number of important unaddressed problems in this rapidly emerging field (Snead and Gladfelter, 2019; Zhang and Rabouille, 2019; Zhao and Zhang, 2020). First, a particularly foundational challenge is our limited understanding of the control of wetting states in cells. Recently, several specific

proteins have been described that allow droplets to wet membranes: Vac8 induces wetting of the autophagosomal PAS droplet at the vacuolar membrane (Fujioka et al., 2020), RNA granules are transported via lysosomes in a manner depending on annexin A11 (Liao et al., 2019), and the ZO proteins ZO1 and ZO2 form droplets that wet plasma membranes (Beutel et al., 2019). Second, posttranslational modifications of phase-separating “scaffold” proteins as well as partitioning “client” molecules are important for droplet formation and stability (Beutel et al., 2019; Fujioka et al., 2020; Milovanovic et al., 2018; Bah et al., 2015; Gerbich et al., 2020), and possibly also for the inherent wettability of droplets. Third, modification of membrane elastic stresses, membrane composition (such as phosphatidylinositol phosphorylation during autophagy and membrane phase separation), and partitioning of lipid binding and curvature-inducing proteins into droplets can together provide a means of fine-tuning organelle and organelle subdomain specific wettability. Indeed, coupling between droplet and membrane phase separation has been demonstrated (Andes-Koback and Keating, 2011), suggesting that distinct physicochemical properties of wetted membrane segments may provide a basis for assembly and stability of membrane domains. These intriguing problems remain to be addressed in future systematic studies.

Another important question is whether droplet-membrane interplay can provide further dynamic spatiotemporal organization of cellular materials. As an example, membrane deformation by droplets could allow for long-range elastic interactions between droplets. Wetting also has the potential to critically influence interactions between molecules within droplets and those bound to intracellular membranes, and indeed those in the surrounding solution. Droplets enclosed by membrane buds, for example, are characterized by a strongly restricted diffusive exchange of molecules across the droplet surface (Agudo-Canalejo et al., 2021).

In addressing these questions, a large number of distinct parameters that influence droplet-membrane interactions must be examined. To this end, robust *in vitro* models will need to be developed as precise quantification of tension and droplet wetting properties is difficult in living systems. A particularly important parameter is the contributions of specific and nonspecific interactions to wetting, which are much easier to determine in controlled *in vitro* systems. In addition, *in vitro* work promises to allow better characterization of surface tensions and contact angles that also will enhance our understanding of intracellular wetting phenomena. Such approaches are well-established in the materials and engineering sciences, but the systematic assessment of cellular material properties, including surface tensions and contact angles, is still in its infancy. Encouragingly, the findings of *in vivo* studies so far suggest that physicochemical characteristics of wetting are broadly comparable to synthetic systems.

We anticipate that a plethora of new elastocapillary phenomena in cells will be discovered in the near future. To make sense of this exciting field, it is essential to commit to an interdisciplinary approach, drawing on biological and physical techniques that employ both *in vivo* and *in vitro* experimentation

as well as mathematical modeling and computer simulations. Continued interrogation of the role of wetting in cells promises to uncover a yet unappreciated physical determinant of intracellular dynamics and organization, enhancing our fundamental understanding of cell biology.

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